

WHAT IS CLAIMED IS:

1. A method for detecting an interaction between a first test agent and a second test agent, comprising:

5 providing a first fusion construct and a second fusion construct, said first fusion construct having an N-intein and said first test agent, said second fusion construct having a C-intein and said second test agent, wherein at least one of the two fusion constructs has an inactive reporter capable of being converted to an active reporter upon trans-  
splicing through said N-intein and said C-intein, and wherein said N-intein and said C-  
10 intein do not interact with each other;

allowing said first test agent in said first fusion construct to interact with said second test agent in said second fusion construct in a substantially cell free environment;  
and

15 detecting said active reporter.

2. The method of Claim 1, wherein said first fusion construct comprises a first inactive reporter fused to the N-terminus of said N-intein.

3. The method of Claim 2, wherein said inactive reporter is a non-  
20 proteinaceous moiety fused to the N-terminus of said N-intein through an amino acid linker.

4. The method of Claim 2, wherein the first test agent is fused to the C-  
terminus of said N-intein.

5. The method of Claim 2, wherein the first test agent is covalently linked to the first inactive reporter.

6. The method of Claim 2, wherein said second fusion construct comprises a  
30 second inactive reporter fused to the C-terminus of said C-intein, and wherein an active reporter is formed upon ligation of said first and second inactive reporters.

7. The method of Claim 6, wherein said second inactive reporter is a non-proteinaceous moiety fused to the C-terminus of said C-intein through an amino acid linker selected from the group consisting of cysteine, serine, and threonine.

8. The method of Claim 6, wherein the second test agent is fused to the N-terminus of said C-intein.

9. The method of Claim 6, wherein the second test agent is covalently linked to said second inactive reporter.

10. The method of Claim 1, wherein said active reporter is detected based on molecular weight.

11. The method of Claim 1, wherein said active reporter is detected by a color assay.

12. The method of Claim 11, wherein said active reporter protein is selected from the group consisting of  $\beta$ -galactosidase, luciferase, green fluorescence protein, blue fluorescence protein, alkaline phosphatase, horseradish peroxidase, and derivatives thereof.

13. A method for detecting protein-protein interaction, comprising:  
providing a first fusion protein and a second fusion protein, said first fusion protein having a first test polypeptide and a first inactive reporter fused to the N-terminus of an N-intein, said second fusion protein having a second test polypeptide and a second inactive reporter fused to the C-terminus of a C-intein, wherein the ligation of said N-intein and C-intein upon trans-splicing results in the formation of an active reporter protein;

mixing said first and second fusion proteins in a substantially cell free environment; and

detecting said active reporter protein.

14. The method of Claim 13, wherein said active reporter protein is detectable by a color assay.

15. The method of Claim 13, wherein said active reporter protein is selected from the group consisting of  $\beta$ -galactosidase, luciferase, green fluorescence protein, blue fluorescence protein, alkaline phosphatase, horseradish peroxidase, and derivatives thereof.

16. A method for detecting protein-protein interaction, comprising:  
providing a protein microarray having a plurality of prey fusion proteins immobilized to a solid substrate, each of said prey fusion proteins having a prey polypeptide and a first inactive reporter fused to the N-terminus of an N-intein;  
contacting said protein microarray with a bait fusion protein having a bait polypeptide and a second inactive reporter fused to the C-terminus of a C-intein, wherein the ligation of said first inactive reporter and said second inactive reporter upon trans-splicing mediated by said N-intein and said C-intein results in the formation of an active reporter protein; and  
detecting said active reporter protein.

17. The method of Claim 16, wherein the prey polypeptide is fused to the N-terminus of said first inactive reporter.

18. The method of Claim 16, wherein the prey polypeptide is fused to the C-terminus of said N-intein.

19. A method for detecting protein-protein interaction, comprising:  
providing a protein microarray having a plurality of prey fusion proteins immobilized to a solid substrate, each of said prey fusion proteins having a prey polypeptide and a first inactive reporter fused to the C-terminus of a C-intein;

contacting said protein microarray with a bait fusion protein having a bait polypeptide and a second inactive reporter fused to the N-terminus of an N-intein, wherein the ligation of said N-intein and C-intein upon trans-splicing results in the formation of an active reporter protein; and

5 detecting said active reporter protein.

20. The method of Claim 19, wherein the prey polypeptide is fused to the C-terminus of said first inactive reporter.

10 21. The method of Claim 19, wherein the prey polypeptide is fused to the N-terminus of said C-intein.

22. A method for detecting protein-protein interaction, comprising:  
expressing a first fusion protein in a first host cell, said first fusion protein having  
15 a signal peptide, a first test polypeptide, and a first inactive reporter fused to the N-terminus of an N-intein, said first fusion protein being secreted from said first host cell;  
expressing a second fusion protein in a second host cell, said second fusion protein having a signal peptide, a second test polypeptide, and a second inactive reporter fused to the C-terminus of a C-intein, said second fusion protein being secreted from said  
20 second host cell, wherein the ligation of said first inactive reporter and said second inactive reporter upon trans-splicing mediated by said N-intein and said C-intein results in the formation of an active reporter protein;  
co-culturing said first host cell secreting said first fusion protein and said second host cell secreting said second fusion protein; and  
25 detecting said active reporter protein.

23. A method for selecting compounds capable interfering with an interaction between a first test agent and a second test agent, comprising:  
providing a first fusion construct and a second fusion construct, said first fusion  
30 construct having an N-intein and said first test agent, said second fusion construct having a C-intein and said second test agent, wherein at least one of the two fusion constructs

has an inactive reporter capable of being converted to an active reporter upon trans-splicing through said N-intein and said C-intein, and wherein said N-intein and said C-intein do not interact with each other;

allowing said first test agent in said first fusion construct to interact with said  
5 second test agent in said second fusion construct in a substantially cell free environment and in the presence of one or more test compounds; and  
detecting said active reporter.

24. A method for selecting compounds capable of interfering with a protein-  
10 protein interaction, comprising:

providing a first fusion protein and a second fusion protein, said first fusion  
protein having a first test polypeptide and a first inactive reporter fused to the N-terminus  
of an N-intein, said second fusion protein having a second test polypeptide and a second  
inactive reporter fused to the C-terminus of a C-intein, wherein the ligation of said first  
15 inactive reporter and said second inactive reporter upon trans-splicing mediated by said  
N-intein and said C-intein results in the formation of an active reporter protein;

mixing said first and second fusion proteins in a substantially cell free  
environment and in the presence of one or more test compounds; and  
20 detecting said active reporter protein.

25. The method of Claim 24, wherein said active reporter protein is detectable  
by a color assay.

26. The method of Claim 24, wherein said active reporter protein is selected  
25 from the group consisting of  $\beta$ -galactosidase, luciferase, green fluorescence protein, blue  
fluorescence protein, alkaline phosphatase, horseradish peroxidase, and derivatives  
thereof.

27. A method for selecting compounds capable of interfering with a protein-  
30 protein interaction, comprising:

providing a protein microarray having a plurality of prey fusion proteins immobilized to a solid substrate, each of said prey fusion proteins having a prey polypeptide and a first inactive reporter fused to the N-terminus of an N-intein;

contacting said protein microarray, in the presence of one or more test  
5 compounds, with a bait fusion protein having a bait polypeptide and a second inactive reporter fused to the C-terminus of a C-intein, wherein the ligation of said first inactive reporter and said second inactive reporter upon trans-splicing mediated by said N-intein and said C-intein results in the formation of an active reporter protein; and  
detecting said active reporter protein.

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28. The method of Claim 27, wherein the prey polypeptide is fused to the N-terminus of said first inactive reporter.

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29. The method of Claim 27, wherein the prey polypeptide is fused to the C-terminus of said N-intein.

30. A method for selecting compounds capable of interfering with a protein-protein interaction, comprising:

providing a protein microarray having a plurality of prey fusion proteins  
20 immobilized to a solid substrate, each of said prey fusion proteins having a prey polypeptide and a first inactive reporter fused to the C-terminus of a C-intein;

contacting said protein microarray, in the presence of one or more test  
compounds, with a bait fusion protein having a bait polypeptide and a second inactive  
reporter fused to the N-terminus of an N-intein, wherein the ligation of said first inactive  
25 reporter and said second inactive reporter upon trans-splicing mediated by said N-intein and said C-intein results in the formation of an active reporter protein; and  
detecting said active reporter protein.

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31. The method of Claim 30, wherein the prey polypeptide is fused to the C-terminus of said first inactive reporter.

32. The method of Claim 30, wherein the prey polypeptide is fused to the N-terminus of said C-intein.

33. A method of selecting compounds capable of interfering with a protein-  
5 protein interaction, comprising:

expressing a first fusion protein in a first host cell, said first fusion protein having a signal peptide, a first test polypeptide, and a first inactive reporter fused to the N-terminus of an N-intein, said first fusion protein being secreted from said first host cell;

10 expressing a second fusion protein in a second host cell, said second fusion protein having a signal peptide, a second test polypeptide, and a second inactive reporter fused to the C-terminus of a C-intein, said second fusion protein being secreted from said second host cell, wherein the ligation of said first inactive reporter and said second inactive reporter upon trans-splicing mediated by said N-intein and said C-intein results in the formation of an active reporter protein;

15 co-culturing said first host cell secreting said first fusion protein and said second host cell secreting said second fusion protein in the presence of one or more test compounds; and

detecting said active reporter protein.